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# Method for Quantitative Determination of Spatial Polymer Distribution in Alginate Beads Using Raman Spectroscopy

MATTHIAS HEINEMANN,\* HOLGER MEINBERG, JOCHEN BÜCHS,  
HANS-JÜRGEN KOB, and MARION B. ANSORGE-SCHUMACHER†

*RWTH Aachen University, Biochemical Engineering (M.H., J.B.), Technical Thermodynamics (H.M., H.-J.K.), and Biotechnology (M.B.A.-S.), 52056 Aachen, Germany*

A new method based on Raman spectroscopy is presented for non-invasive, quantitative determination of the spatial polymer distribution in alginate beads of approximately 4 mm diameter. With the experimental setup, a two-dimensional image is created along a thin measuring line through the bead comprising one spatial and one spectral dimension. For quantitative analysis of the Raman spectra, the method of indirect hard modeling was applied to make use of the information contained in the entire recorded spectra. For quantification of the alginate signals from within the beads, a calibration curve acquired from sodium alginate solutions was used after it was shown that only negligible differences occur between signals from alginate solutions and alginate gels. The distribution of alginate over the bead gel matrix was acquired with high spatial (51  $\mu\text{m}$ ) and time (12 s) resolution. The inhomogeneous distribution obtained using the new measuring technique is qualitatively in excellent agreement with data from the literature. In contrast to known measuring techniques, correct quantitative information about the spatial polymer distribution within the matrix was derived. It gave an alginate mass fraction of approximately 0.045 g/g at the edges and 0.02 g/g in the center of the beads. Next to the determination of mere polymer concentrations, the excellent time resolution of the presented method will enable investigation of the dynamic process of gel formation and it will also serve as a basis for investigation of mass transfer of small diffusing molecules in alginate matrices.

**Index Headings:** Raman spectroscopy; Alginate bead; Hydrogel; Non-invasive measurement; Quantification; Polymer distribution; Indirect hard modeling.

## INTRODUCTION

Hydrogels are cross-linked three-dimensional macromolecular networks that contain a large fraction of water within their structure. Besides their application in the food sector for structuring of products,<sup>1,2</sup> they have found application in many biomedical, pharmaceutical, and technical areas, for example, as natural tissue,<sup>3,4</sup> as systems for drug delivery and controlled release,<sup>3</sup> as carriers for biocatalyst immobilization,<sup>5</sup> or as absorbant particles in chromatographic processes.<sup>6</sup> One of the most important properties of hydrogels leading to this broad versatility is their ability for controlled uptake, release, or retention of molecules. This ability, in turn, is due to specific interactions of the macromolecular network with the diffusing or retained molecule. The extent of these interactions, however, strongly depends on the polymer concentration<sup>7</sup> and on the polymer's chemical nature.<sup>8,9</sup> Consequently, transport processes within the hydrogel matrix

such as diffusion, which are of profound interest for most areas of application, cannot be studied without taking into account the true local polymer concentration within the hydrogel. The knowledge of the local polymer distribution is especially required when alginate hydrogel matrices are studied. Inside these kinds of gel matrices, significant polymer gradients occur with considerably lower concentration in the center of the gel than at the edges due to a particular gelling process.<sup>10</sup>

Taking into account the local alginate concentration, the diffusion of polyethylene glycols in heterogeneous alginate gel cylinders was recently investigated by Kwak and Lafleur.<sup>11</sup> They employed Raman spectroscopy to measure both the concentration profile of the diffusant and the local polymer concentration. However, despite the fundamental character of this work, some drawbacks are obvious: First, only cylindrically shaped gels, fitted into Raman glass tubes smaller than those used for the formation of the gels, were investigated, risking a deformation or even local disruption of the gel structure and thus, a poorly reproducible polymer distribution. Also, for most applications, alginate gels are employed as beads and as the alginate distribution is significantly influenced by the shape, size, and production process of the alginate gels,<sup>12</sup> a technique for the investigation of beads would be more suitable. Besides, the technique developed by Kwak and Lafleur<sup>11</sup> only allowed a point-wise measurement. Thus, to obtain concentration profiles from within the gel, the laser had to be moved from point to point. Due to the employed extended measurement time (approximately 1 h), only a low spatial resolution (approximately 24 mm) was chosen in the considered diffusion experiment. Although this spatial and time resolution was sufficient for studying the transport of the slowly diffusing polyethylene glycols, it is most likely that a higher spatial and time resolution is required when diffusion of low molecular weight compounds is investigated.

In microscopically small gel structures, higher spatial resolutions can be obtained when a Raman spectroscopy setup is coupled to a confocal microscope. In this case, spatial resolutions of approximately 2  $\mu\text{m}$  were obtained on the micrometer-length scale.<sup>13–15</sup>

In this work, however, we focus on the macroscopic level and present a new method for a quantitative determination of the spatial polymer distribution in alginate gels (on the scale of a few millimeters) as a basis for investigations on mass transfer of small diffusing molecules in hydrogels. The developed method is also based on Raman spectroscopy, but it overcomes the outlined limitations of the method developed by Kwak and La-

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\* Present address: ETH Zürich, Institute of Process Engineering, 8092 Zürich, Switzerland.

† Author to whom correspondence should be sent. E-mail: m.ansorge@biotec.rwth-aachen.de.

fleur<sup>11</sup> with respect to the measurement in beads and to spatial and time resolution. Additionally, instead of using intensities at single wave numbers to obtain quantitative information, we analyze the complete recorded spectrum, resulting in an improved quantification of the polymer concentration.

## EXPERIMENTAL

**Purification of Alginate.** It is known that commercial sodium alginate can contain cell fragments, insoluble alginic acid, metal ions, etc.<sup>16</sup> The amount and type of impurities strongly depend on the origin, composition, and production process of the respective sodium alginate.<sup>17</sup> Thus, before the employed Manugel® DJX sodium alginate (Monsanto, Waterfield, UK) was used to produce beads, it was purified according to the following procedure: 5 g alginate were dissolved in a solution containing 30 mL deionized water and 40 mL tertiary butanol (*purum*; Fluka, Buchs, Switzerland). After stirring over night, the solution was filtered with a glass fiber filter (MN 615, Macherey-Nagel, Düren, Germany). The filter residue was dissolved in deionized water to give an alginate solution of about 20 g/L. This solution was subsequently filtered with a nylon membrane with a glass microfiber prefilter (PolyCap® 75 AS, Whatman, Clifton, NJ). Finally, an aqueous solution of the filter residue was lyophilized. This procedure removed the yellowish color from the alginate preparation and yielded alginate gels with significantly improved transparency and therefore reduced fluorescence in Raman spectroscopic measurements.

**Water Content of Purified Alginate Preparation.** Solid (lyophilized) sodium alginate is known to hold significant amounts of bound water.<sup>16</sup> In order to obtain a valid quantification of the alginate distribution in the alginate bead, the water content of the purified alginate preparation was determined thermogravimetrically with a ThermoAnalyser 2000C (Mettler, Greifensee, Switzerland). By keeping a sample of purified sodium alginate at 150 °C for 30 min, a weight loss of 10% occurred, revealing a water content of 10%. This is in general agreement with information from the supplier: An average water content of about 13% was given for different sodium alginate preparations at standard humidity and room temperature.

**Alginate Bead Production.** Two grams of purified sodium alginate was dissolved in 98 g deionized water to give a homogeneous solution with an alginate mass fraction (defined as mass of alginate divided by the total mass of the mixture) of 0.018 g/g when taking into account the water content of the alginate preparation. Almost exactly spherical beads with a diameter of approximately 3.8 mm were produced according to a recently developed method for external gelation<sup>18</sup> by dropping 50 µL of the alginate solution into a cylinder containing the four solvents *n*-hexane, 1-butanol, 1-butanol with CaCl<sub>2</sub> (1% w/v), and deionized water with CaCl<sub>2</sub> (2% w/v). The excellent spherical shape of the beads obtained with this method allows for a later transfer of the data obtained from the one-dimensional measurement to the complete three-dimensional bead.

During gel formation, a shrinkage of the bead occurs,

which results in an increased alginate mass fraction in the bead<sup>19–21</sup> due to a loss of water. After gelation, the mean alginate mass fraction in the bead,  $w_A^f$ , can be calculated from

$$w_A^f = \frac{V_{\text{drop}} \cdot w_A^i}{\frac{4}{3} \cdot \pi \cdot r^3} \quad (1)$$

using the volume of the alginate solution dropped into the solvent cylinder,  $V_{\text{drop}}$  (50 µL), the alginate mass fraction of this solution,  $w_A^i$  (0.018 g/g), and the radius of the bead,  $r$ , which is obtained from the Raman spectroscopic measurement with the assumption that the edge of the bead corresponds with the highest alginate signal. Even over several hours, a change in size of the bead was not observed.

**Experimental Setup.** The layout of the experimental equipment, which is mounted on an anti-vibration table, is shown in Fig. 1. A focused beam of an argon ion laser (Spectra Physics, Darmstadt, Germany) is directed through an alginate bead using an excitation wavelength of 514.5 nm and a power of 450 mW. The bead is placed on a black anodized holder (not shown in Fig. 1) situated in a quartz cell (inner dimensions 10 × 10 × 40 mm), which is filled with 3 mL deionized water. The quartz cell containing the bead is mounted on a micro-positioning stage (x, y, z-direction), which allows the cell to be positioned in a way that the laser is directed to the center of the bead without any diffraction of the laser beam inside the bead. With a telescope consisting of two lenses ( $f = 200$  mm,  $f = -20$  mm), the diameter of the laser beam was reduced to 100 µm to get a measuring line, from which spectra are being registered. With our setup, this measuring line comprises the complete alginate bead and also parts of the liquid above and below the bead.

The Raman signals emitted along the measuring line are collected by a macro-lens (Nikon Nikkor 200 mm 1:4D, Japan) at an angle of 90°. The signal is then cleared up by a Raman holographic notch filter assembly (Acton Research Corporation, NFC-446-040, Acton, MA), including two acromat lenses and micrometer control for tilt-tuning of a holographic notch filter (Kaiser Optical Systems, Ann Arbor, MI) with a narrow bandwidth to eliminate the much more intensive scattered radiation of the excitation wavelength. The resulting signal was focused towards the entrance slit of the spectrograph (Acton Research Corporation, Spectra Pro 500i, Acton, MA).

The grating of the spectrograph with 1200 lines/mm resolves the first-order diffraction into a spectrum without distorting the one-dimensional image of the measuring line. The two-dimensional image created in this way (one spatial and one spectral dimension) is recorded by a thermoelectrically cooled charge-coupled device camera (Roper Scientific NTE/CCD-1340/400, Acton, MA). The photosensitive chip (back-illuminated) has an efficiency of about 90% (conversion from a photon to an electron) in the considered wavelength range of 500 to 600 nm. Every row of the CCD chip, a total of 400, is collecting the Raman spectrum from a small segment of the one-dimensional measuring line. For the investigation of the alginate beads, in each case, two rows were binned to increase the signal-to-noise ratio, leading to 200 rows

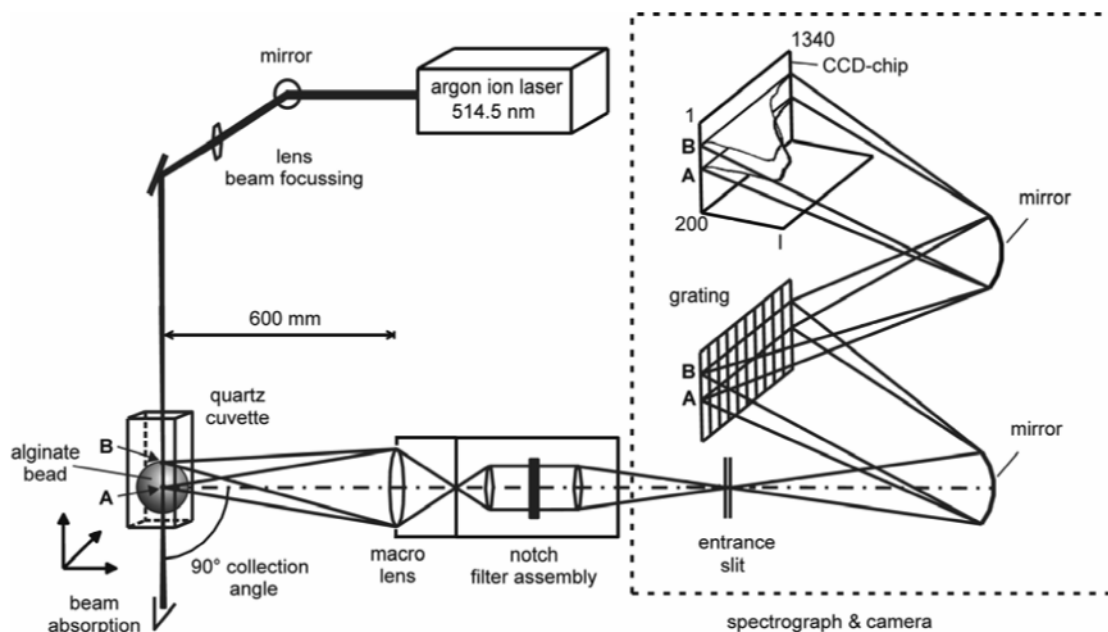


FIG. 1. Experimental setup.

with a spatial resolution of  $51 \mu\text{m}/\text{row}$ . The photosensitive chip is fixed with an angle of  $9^\circ$  to the spectrograph, which, in preliminary experiments, has been shown to be the best compromise between spatial and spectral resolution.

The excellent transparency of the alginate beads accounted for minimized energy absorption and consequently minimized fluorescence. Thus, only a very slight temperature increase ( $0.5^\circ\text{C}$ ) was observed in the water phase around the bead after having exposed the system to the laser with a power of 450 mW for one hour. Because the O–H stretching vibration bands of the spectra obtained from within the bead also did not show changes in form and position, which are known to be very sensitive to temperature,<sup>22</sup> energy absorption was shown to be negligible, especially as in the actual experiments the exposure time was only 12 s. Thus, all the data presented within this work refer to data at  $24^\circ\text{C}$  ( $\pm 0.5^\circ\text{C}$ ).

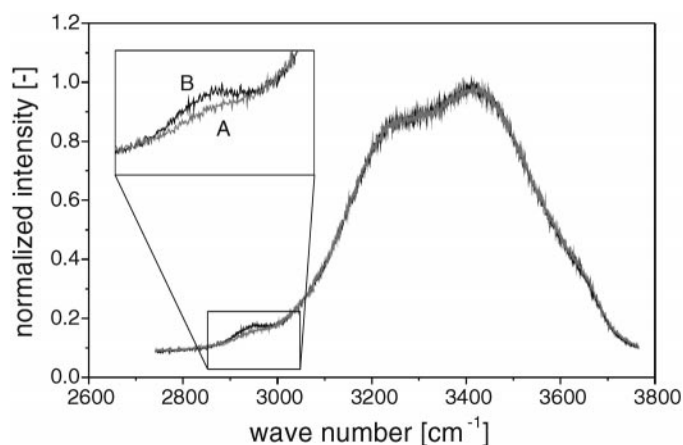


FIG. 2. Typical Raman spectra from (A) the center and (B) the edge of Ca-alginate beads. The intensities have been normalized relative to the maximum of the O–H band.

## SPECTRA ANALYSIS AND CALIBRATION

**Spectra Analysis.** Spectra were recorded between  $2740\text{--}3765 \text{ cm}^{-1}$ . In this range, spectra from alginate beads show two characteristic bands: a very broad and intensive band of the O–H stretching vibration of water with a maximum around  $3400 \text{ cm}^{-1}$  and a small broad band of the C–H stretching vibrations of the alginate around  $2938 \text{ cm}^{-1}$ . Figure 2 shows two typical spectra, one from the center of the alginate bead (spectrum A) and one from the top edge of the alginate bead (spectrum B). It can be seen from these spectra that the signal from the alginate is very weak compared to the strong water signal. Nevertheless, it is obvious that the alginate is not homogeneously distributed in the bead.

In order to discriminate the overlapping signals coming from the different substances, a computational technique for spectra analysis called “indirect hard modeling” was used.<sup>23</sup> Here, the experimental spectrum of a mixture was fitted by the scaled superposition of spectra, which are related to each substance. The obtained scaling factors correspond to the masses of the two substances, water and alginate. In contrast to classical least square methods (CLS), these spectra do not consist of experimental data points but are synthesized from Gaussian, Lorentzian, and Voigt functions with parameters for positions, line widths, and relative heights. These functions are fitted to the experimental spectra to give minimal differences between the synthetic spectrum and the experimental one. After finding a suitable parameter set for each substance, these sets are kept constant with the exception of very few selected parameters, which are used to describe possible changes in line shape or position caused by intermolecular interactions or by the influence of temperature. This leads to a very robust evaluation technique because only the scaling factors corresponding to the masses of the substances in the mixture and the few selected parameters have to be fitted. In the case of negligible molecular



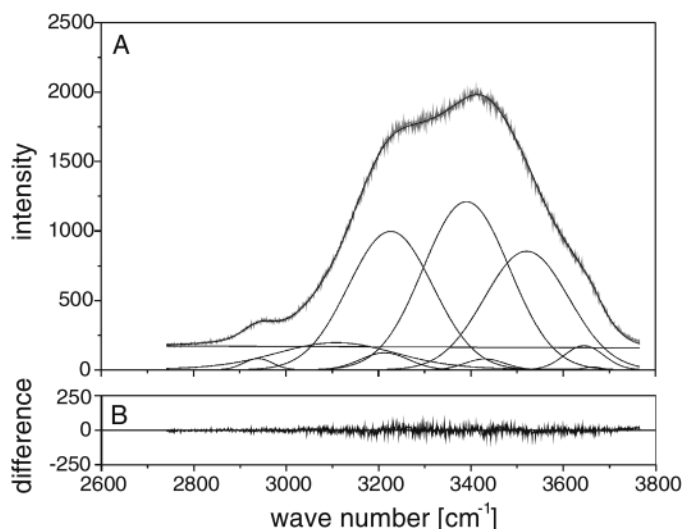


FIG. 3. (A) Typical Raman spectrum of a Ca-alginate bead together with the employed Gaussian, Lorentzian, and Voigt functions for mathematical approximation of the spectrum. (B) Differences between the experimental spectrum and the mathematical model.

interaction, and therefore without the need for fitting selected parameters, this method turns into the common CLS techniques, where only scaling factors must be fitted.

To describe the asymmetrical water band, we use a superposition of ten different functions (compare Fig. 3A) to minimize the difference between the experimental and synthetic spectrum (compare Fig. 3B). In contrast, only a single function was necessary to describe the alginate band. The OH stretching vibrations of the alginate were not taken into account, because we saw no influence from them during spectral analysis. The function describing the alginate band is the one at the very left in Fig. 3A with the maximum at 2938 cm<sup>-1</sup>. The background was fitted with a straight line.

**Calibration.** For calibration, pure water and three different aqueous solutions of sodium alginate (employed alginate mass fractions: 0.013, 0.0261, and 0.0424 g/g) were used. From each of the four solutions, three spectra were recorded by averaging all 400 rows of the chip after excitation with 450 mW and an exposure time of 0.5 s. The spectra from the sodium alginate solutions show the alginate peak at 2932 cm<sup>-1</sup> (data not shown), which is very close to the band of the alginate gel at 2938 cm<sup>-1</sup>. The employed spectra analysis method allows the transfer of the calibration from the solution to the bead by using the same parameter set with the exception of one parameter for line position. Keeping all other parameters constant, especially parameters that correspond to band shapes (alginate solution and alginate gel), resulted in excellent spectral fits.

Aqueous solutions of sodium alginate were used to acquire a calibration curve for quantification of the alginate signals in the gel bead because strictly homogeneous Ca-alginate matrices do not exist. This is evident from the work of Skjåk-Bræk et al.<sup>24</sup> and Draget et al.,<sup>25</sup> who both developed methods to improve the homogeneity of alginate gels. They demonstrated that the homogeneity of alginate gels depends on many factors, especially alginate concentration, which makes the use of differently con-

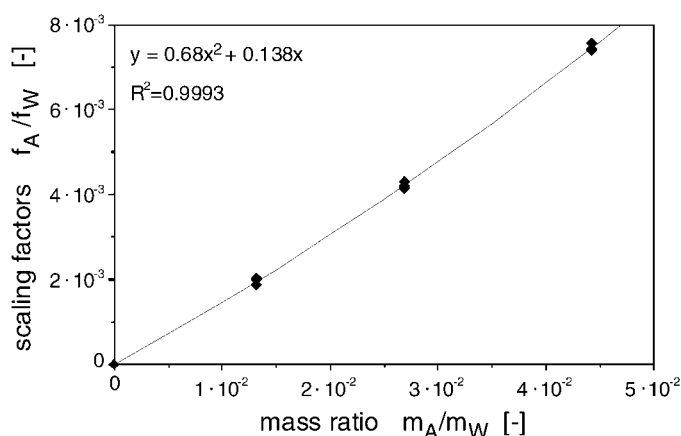


FIG. 4. Calibration curve with each alginate concentration measured in triplicate.

centrated gels for calibration difficult. Using homogeneous solutions instead of “improved” beads prepared according to Skjåk-Bræk et al.<sup>24</sup> as was done by several authors,<sup>26,27</sup> we prevent inaccuracies due to potential failures in alginate homogeneity<sup>28</sup> or due to the (in most cases) quantitatively unknown increase in alginate mass fraction, which is caused by the bead shrinkage occurring during the gelling process.<sup>19–21</sup>

Figure 4 presents the calibration curve obtained from the sodium alginate solutions. Here, the scaling factors of alginate and water are plotted against the corresponding mass ratios (defined as mass of alginate divided by the mass of water). By using ratios instead of absolute values of signal intensities, negative influences of the experimental setup are prevented, such as inhomogeneities of laser power along the measuring line and different measuring volumes caused by wavelength-independent refractive index changes along the light path. The effectiveness of this procedure was proofed by determining the mass ratios along the measuring line in a homogeneous solution, which leads to relative errors less than 1.5%,<sup>29</sup> which may be caused by wavelength-dependent refractive index changes and which can be decreased to 0.2% by using a spatial dependent calibration.<sup>30</sup>

The obtained relationship between the computed scaling factors and the respective mass ratios was described by a polynomial expression. The mass fraction is calculated by

$$w_A = \frac{m_A/m_w}{m_A/m_w + 1}$$

For details, the reader is referred to Bardow et al.<sup>29</sup> The transferability of the calibration curve from alginate solutions to alginate gel is shown in the following section.

## RESULTS AND DISCUSSION

Figure 5 shows alginate mass fraction profiles of five different beads. The inhomogeneous distribution found in our experiment is qualitatively in excellent agreement with the findings of Skjåk-Bræk et al. (1986).<sup>10</sup> By physical sectioning of an alginate gel followed by gravimetric analysis of the slices, these authors found a higher alginate mass fraction at the outer shell of the gel matrix than in the middle. When divalent metal ions such as calcium

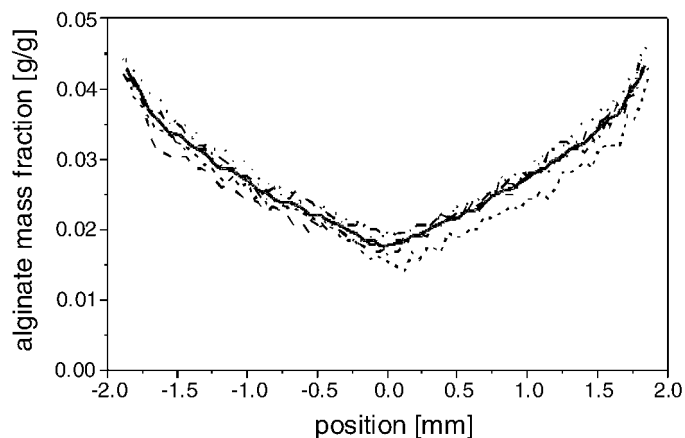


FIG. 5. Alginate mass fraction profiles of five different Ca-alginate beads as a function of the diameter. The thick solid line represents the average alginate distribution.

diffuse into an alginate solution, the rapid ion binding and formation of the polymeric network produced an inwardly moving gelling zone. In fact, alginate moves from the core of the gel towards this gelling zone, leading to a depletion of alginate in the core. The polymer gradient is essentially governed by the relative diffusion rate between the soluble alginate molecules and the gel-forming ion.<sup>12,24</sup> In the following years, this inhomogeneity of alginate gels was verified by several other authors using more sophisticated methods based on magnetic resonance imaging,<sup>26,27</sup> confocal laser scanning microscopy,<sup>28</sup> and most recently also Raman spectroscopy.<sup>11</sup>

Besides the qualitative information, Fig. 5 also provides quantitative information about the spatial polymer distribution. It is obvious from this figure that the mean alginate mass fraction in the bead is higher than the initial mass fraction of the employed sodium alginate solution (0.018 g/g). By using the data presented in Fig. 5, the exact mean alginate mass fraction in the bead was calculated by averaging the data of the two halves of the bead by fitting a polynomial expression of the sixth order to the data and by calculating the integral of the obtained function. After dividing the functional value of the integral by the bead volume, an average alginate mass fraction of 0.035 g/g was obtained. This mass fraction is about 94% higher than the mass fraction of the sodium alginate solution used for bead production. This increase in mass fraction can be explained by a shrinking of the bead during the gelling process, accompanied by a loss of water.<sup>19–21</sup>

Using Eq. 1 with the knowledge of the final bead size, the mean alginate mass fraction results in 0.033 to 0.034 g/g (the range is due to the uncertainty in the determination of the bead diameter). These values are in good agreement with the mean alginate mass fraction obtained by the integration of the calibrated Raman data (0.035 g/g), which justifies our initial assumption of the transferability of the calibration curve from alginate solutions to alginate gel. Additionally, it indicates that the complexation of the  $\text{Ca}^{2+}$  ion with the carboxylate groups of the alginate occurring during gel formation obviously has only a very minor impact on the C–H groups. In summary, these findings confirm that the mass fractions provided for the polymer distribution in the bead are correct.

This is presuming, of course, that the true alginate mass fraction in the applied preparation was correctly determined by taking into account the content of bound water while neglecting residual impurities. In principle, however, quantitative data obtained with the presented Raman spectroscopic method are reliable for any alginate gel prepared from well-characterized or thoroughly purified sodium alginate.

The quantitative data derived from our measurements significantly distinguish themselves from the (rare) quantitative information provided in the literature and obtained with non-invasive techniques. Confocal laser scanning microscopy performed by Strand et al. (2003)<sup>20</sup> and the Raman spectroscopic measurements of Kwak and Lafleur (2003)<sup>10</sup> gave only intensity distributions, which cannot be considered as true quantitative data. With non-invasive techniques, quantitative data were only obtained by Potter et al. (1993)<sup>26</sup> and Thu et al. (2000)<sup>27</sup> by the use of NMR. However, when the NMR measurements were calibrated with signals from assumed homogeneous alginate beads of known initial alginate mass fraction, the shrinkage of the beads during their production and the consequently higher mass fractions of the resulting Ca-alginate beads were not taken into account.

While the technique presented here is capable of studying polymer concentration profiles in macroscopically large objects (on the millimeter scale), other techniques such as confocal Raman spectroscopy<sup>13–15</sup> or Fourier transform infrared (FT-IR) microscopy<sup>31</sup> allow the study of structures on the micrometer level with improved spatial resolution. However, for macroscopically large structures such as the inhomogeneity of 4 mm alginate beads investigated here, a microscopic technique would not have been adequate.

## CONCLUSION

A method based on Raman spectroscopy was presented that allows the non-invasive determination of the spatial polymer distribution in alginate beads. The presented method overcomes various limitations of other methods: a thorough quantification based on a computational analysis of the complete recorded spectral information and an appropriate calibration was achieved, the measurement in beads and matrices of other shapes is feasible, and compared with other methods capable of measuring in macroscopically large structures an excellent spatial and time resolution is possible. If time resolution is not required, data with even further reduced measurement noise can be easily obtained by increasing exposure time.

The time resolution, which is possible with the presented experimental setup, is not important for the determination of alginate profiles itself. However, with the spatial resolution achieved and the possible time resolution, the developed method can later be used for investigating mass transfer of small diffusing molecules in alginate beads and for studying the impact of the polymer matrix on mass transfer. Although, it is not possible to see the different CH stretching vibrations of alginate with this method (which is also not the case with the method developed by Kwak and Lafleur (2003),<sup>11</sup> the method allows the study of the mass transfer of 1-butanol in alginate beads as it is currently done in the authors' lab.

Additionally, the excellent time resolution allows for investigation of the dynamic process of gel formation. Thus, with the presented Raman spectroscopy based measuring technique, investigations of alginate matrices themselves as well as processes within these matrices are possible.

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1. S. M. Clegg, "Thickeners, gels and gelling", in *Physico-chemical aspects of food processing*, S. T. Beckett, Ed. (Blackie Academic & Professional, London, 1995), p. 117.
2. V. B. Tolstoguzov, *Food Hydrocol.* **9**, 317 (1995).
3. S. Dumitriu, "Polysaccharides as biomaterials", in *Polymeric Biomaterials*, S. Dumitriu, Ed. (Marcel Dekker, Inc., New York, 2002), p. 1.
4. A. C. Jen, M. C. Wake, and A. G. Mikos, *Biotechnol. Bioeng.* **50**, 357 (1996).
5. J. K. Park and H. N. Chang, *Biotechnol. Adv.* **18**, 303 (2000).
6. A. Tuncel, E. Ünsal, and S. Senel, "Polymer beads in biomedical chromatography: Preparation and characterization", in *Colloidal Polymers—Synthesis and Characterization*, A. Elaissari, Ed. (Marcel Dekker, Inc., New York, 2003), p. 319.
7. A. Martinsen, I. Storrø, and G. Skjåk-Bræk, *Biotechnol. Bioeng.* **39**, 186 (1992).
8. A. H. Muhr and J. M. V. Blanshard, *Polymer* **23**, 1012 (1982).
9. B. Amsden, *Macromolecules* **31**, 8382 (1998).
10. G. Skjåk-Bræk, O. Smidsrød, and B. Larsen, *Int. J. Biol. Macromol.* **8**, 330 (1986).
11. S. Kwak and M. Lafleur, *Appl. Spectrosc.* **57**, 768 (2003).
12. A. Mikkelsen and A. Elgsaeter, *Biopolymers* **36**, 17 (1995).
13. P. D. A. Pudney, T. M. Hancewicz, and D. G. Cunningham, *Spectrosc. Int. J.* **16**, 217 (2002).
14. P. D. A. Pudney, T. M. Hancewicz, D. G. Cunningham, and M. C. Brown, *Vib. Spectrosc.* **34**, 123 (2004).
15. P. D. A. Pudney, T. M. Hancewicz, D. G. Cunningham, and C. Gray, *Food Hydrocol.* **17**, 345 (2003).
16. W. Diemair and H.-H. Weichel, *Deut. Lebensm.-Rundsch.* **54**, 51 (1958).
17. U. Zimmermann and M. Behringer, German Patent 19,836,960 (2000).
18. A. Buthe, W. Hartmeier, and M. B. Ansorge-Schumacher, *J. Microencapsul.* **21**, 865 (2004).
19. D. Quong, R. J. Neufeld, G. Skjåk-Bræk, and D. Poncelet, *Biotechnol. Bioeng.* **57**, 438 (1998).
20. A. Martinsen, G. Skjåk-Bræk, and O. Smidsrød, *Biotechnol. Bioeng.* **33**, 79 (1989).
21. N. M. Velings and M. M. Mestdag, *Polym. Gels Netw.* **3**, 311 (1995).
22. M. Becucci, S. Cavalieri, R. Eramo, L. Fini, and M. Materazzi, *Laser Phys.* **9**, 422 (1999).
23. F. Alsmeyer, H.-J. Koß, and W. Marquardt, *Appl. Spectrosc.* **58**, 975 (2004).
24. G. Skjåk-Bræk, H. Grasdalen, and O. Smidsrød, *Carbohydr. Polym.* **10**, 31 (1989).
25. K. I. Draget, K. Ostgaard, and O. Smidsrød, *Carbohydr. Polym.* **14**, 159 (1991).
26. K. Potter, T. A. Carpenter, and L. D. Hall, *Carbohydr. Res.* **246**, 43 (1993).
27. B. Thu, O. Gaserod, D. Paus, A. Mikkelsen, G. Skjåk-Bræk, R. Toffanin, F. Vittur, and R. Rizzo, *Biopolymers* **53**, 60 (2000).
28. B. L. Strand, Y. A. Morch, T. Espevik, and G. Skjåk-Bræk, *Biotechnol. Bioeng.* **82**, 386 (2003).
29. A. Bardow, W. Marquardt, V. Göke, H. J. Koss, and K. Lucas, *AIChE J.* **49**, 323 (2003).
30. A. Bardow, V. Göke, H.-J. Koß, K. Lucas, and W. Marquardt, *Fluid Phase Equilib.*, paper in press (2004).
31. C. M. Durrani and A. M. Donald, *Carbohydr. Polym.* **28**, 297 (1995).